

3' non-translated sequences in *Drosophila* cyclin B transcripts direct posterior pole accumulation late in oogenesis and peri-nuclear association in syncytial embryos

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Summary

We have characterised forms of the *Drosophila* cyclin B transcript that differ as a result of a splicing event which removes a nucleotide segment from the 3' untranslated region. In oogenesis, both cyclin A RNA and a shorter form of the cyclin B transcript are seen in the cells of the germarium that are undergoing mitosis. The shorter cyclin B transcript alone is then detectable in the presumptive oocyte until stages 7-8 of oogenesis. Both cyclin A RNA and a longer form of the cyclin B RNA are then synthesised in the nurse cells during stages 9-11, to be deposited in the oocyte during stages 11-12. These transcripts become evenly distributed throughout the oocyte cytoplasm but, in addition, those of cyclin B become con-

centrated at the posterior pole. Examination of the distributions of RNAs transcribed from chimeric cyclin genes indicates that sequences in the 3' untranslated region of the larger cyclin B RNA are required both for it to become concentrated at the posterior pole and to direct those transcripts in the body of the syncytial embryo to their peri-nuclear localisation. These sequences are disrupted by the splicing event which generates smaller cyclin B transcripts.

Key words: cyclin B, *Drosophila*, posterior pole, RNA localisation, oogenesis.

Introduction

The mitotic cyclins, proteins first identified in the eggs of marine invertebrates, are characterised by changes in their abundance during the cell cycle; they accumulate in interphase and are abruptly degraded in mitosis (Evans et al., 1983; Standart et al., 1987; Swenson et al., 1986). Cyclins are required to complex with and thus activate the major cell cycle regulatory protein kinase p34^{cdc2} (Swenson et al., 1987; Draetta et al., 1989; Meijer et al., 1989; Solomon et al., 1990; Parker et al., 1991; Gautier and Maller, 1991). The active kinase complex brings about the phosphorylation of a variety of substrates including histone H1, microtubules, centrosomes and nuclear lamins. These phosphorylation events are among those required for chromosome condensation, organisation of the mitotic spindle and breakdown of the nuclear envelope prior to entry into mitosis (reviewed by Moreno and Nurse, 1990). Inactivation of the p34^{cdc2} kinase is mediated by abrupt degradation of the cyclins. Addition of a cyclin B mRNA encoding a truncated form of the protein, resistant to proteolytic cleavage, prevents exit from mitosis in *Xenopus* oocyte extract showing that cyclin degradation is necessary for progress through the cell cycle (Murray and Kirschner, 1989; Murray et al., 1989).

p34^{cdc2} is part of a conserved mechanism that regulates

the G2-M transition. Thus, the *cdc2* gene product of *S.pombe* may be functionally replaced by the *Saccharomyces cerevisiae*, *Drosophila* or human homologues (Beach et al., 1982; Jimenez et al., 1990; Lehner and O'Farrell, 1990b; Lee and Nurse, 1987). The A and B type cyclin genes are similarly conserved and have been cloned and sequenced from a number of higher eukaryotes including *Drosophila*, sea urchins, clams, *Xenopus* and humans (Lehner and O'Farrell, 1989, 1990a; Whitfield et al., 1989; Pines and Hunt, 1987; Swenson et al., 1986; Westendorf et al., 1989; Minshull et al., 1989, 1990; Pines and Hunter, 1989, 1990). A comparison of the cyclin sequences in a wide range of organisms points towards the conservation of the distinct A and B types of cyclin suggesting these molecules have differing roles in the cell cycle. In *Drosophila* this is supported by the finding that embryos having mutations in the cyclin A gene arrest development in the cell cycles that follow cellularisation, once the maternal contribution to the embryo has been exhausted. Hence cyclin B cannot substitute for cyclin A function (Lehner and O'Farrell, 1989). Examination of the behaviour of cyclin A and B proteins in cellularised *Drosophila* embryos and larval brains reveals differences in the timing of their accumulation and breakdown (Whitfield et al., 1990). This may reflect the differential timing of the activation of the p34^{cdc2} kinase associated with either cyclin A or cyclin B

as observed in *Xenopus* cell-free systems (Minshull et al., 1990). Pines and Hunter (1991) have also found that the two cyclins accumulate in different sub-cellular compartments in mammalian cells. In the syncytial *Drosophila* embryo, cyclin A appears to shuttle between an association with chromatin and the cytoplasm, whereas cyclin B is localised to the region at which nuclear envelope breakdown begins and is subsequently associated with polar microtubules of the mitotic spindle (Maldonado-Codina and Glover, 1992).

An abundant maternal supply of both cyclin A and B transcripts is present in the unfertilized *Drosophila* egg. Unlike the uniformly distributed cyclin A transcripts, those of cyclin B are concentrated at the posterior pole of egg. During embryogenesis cyclin B mRNA becomes incorporated into the progenitors of the germ-line, the pole-cells (Whitfield et al., 1989; Lehner and O'Farrell, 1990a; Raff et al., 1990). Cyclin B transcripts also become more closely concentrated around the somatic nuclei than those of cyclin A in a manner that requires the integrity of microtubules. In order to establish and maintain the posterior pole localisation, a component of the posterior cytoplasm is required. The distribution of cyclin B transcripts in a variety of mutant embryos that fail to form pole cells suggests that this localisation requires a polar granule associated component (Raff et al., 1990). In this paper we examine the distribution patterns of cyclin A and B transcripts during oogenesis and identify a signal in the cyclin B transcript required for both the posterior pole and nuclear localisation.

Materials and methods

Northern hybridisation

Hybridisation was to poly (A)⁺ RNA immobilised on nitrocellulose filters (gift of Dr. K. O'Hare) with probes made by random oligo-labelling (Feinburg and Vogelstein, 1983) of cDNA fragments shown in Fig 2. Hybridisation was carried out at 42°C for 24 hours in 50% formamide, 0.75 M NaCl, 0.15 M Tris-HCl pH 8, 10 mM EDTA, 200 µg/ml denatured salmon sperm DNA, 0.5% SDS, 36 mM Na₂HPO₄, 4 mM NaH₂PO₄, 5 × Denhardt's solution. Filters were washed in 5 × Denhardt's, 0.3 M NaCl, 60 mM Tris-HCl pH 8, 4 mM EDTA, 2 × 30 minutes followed by 75 mM NaCl, 15 mM Tris-HCl, 1 mM EDTA, 3 × 10 minutes. Filters were exposed for 7 days at -70°C with intensifying screens.

In situ hybridisation to whole mount embryos and ovaries

In situ hybridisation was carried out according to the method of Tautz and Pfeifle (1989). Ovaries were dissected in 0.7% NaCl and placed directly into fixative (4% paraformaldehyde in PBS; phosphate buffered saline, 120 mM NaCl, 10 mM sodium phosphate, with an equal volume of heptane). Embryos were also treated using this fixative after dechorionation in 50% hypochlorite. Following fixation, embryos were devitellinised by vigorous shaking in 1:1 heptane:methanol (Mitchison and Sedat, 1983) and then pre-treated for hybridisation as described by Tautz and Pfeifle. Appropriate DNA fragments cloned into the vector pKS Bluescript (Stratagene) were used as templates to transcribe digoxigenin-labelled antisense RNA probes from either the T3 or T7 promoters for detection of the chimeric transcripts. Probes were

also transcribed from 2.5kb cyclin A and 2.7kb cyclin B cDNA templates linearised with *Sall* and *HindIII* respectively, using the T7 promoter in the vector pNB40 (Brown and Kafatos, 1988) for detection of the endogenous cyclin A and B transcripts in ovaries. The cyclin B 3' end probe was transcribed from the 2.7kb cyclin B cDNA template linearised with *EcoRV*. Detection of hybridisation was by an alkaline phosphatase conjugated anti-digoxigenin antibody. Probe synthesis and detection were performed using BCL RNA labelling and nucleic acid detection kits. Prior to hybridisation, all probes were reduced in size by alkaline hydrolysis in 40 mM NaHCO₃, 60 mM Na₂CO₃, pH 10, for 2 hours at 60°C. Probes were precipitated and resuspended in 50 µl water. For each hybridisation 10 µl of probe was added to embryos or ovaries in approx. 90 µl hybridisation solution. Hybridisations were carried out at 47°C for 16 hours. Hybridisation solution and subsequent treatments as Tautz and Pfeifle, 1989. All tissue was dehydrated through an ethanol series prior to mounting in 1.6 g/ml Canada balsam in methyl salicylate. For visualisation of DNA embryos were stained for 20 minutes in 10 µg/ml Hoechst 33258 and mounted in 85% glycerol, 12.5% water, 2.5% propylgallate. Photography was carried out using a Nikon Microphot under bright field illumination.

In situ hybridisation to sectioned ovaries

Ovaries were dissected in 0.7% NaCl and placed directly into fixative as above. After 2 hours fixation ovaries were dehydrated through an ethanol series and pre-treated for embedding and sectioning as described for embryos in Ingham et al., (1985). 15 µm paraffin sections, cut using a Biotuc microtome, were air dried and stored at 4°C until use. Antisense RNA probes were prepared in the same way as for use in whole mount in situ hybridisation. Hybridisation conditions were as described in Ingham et al., 1985. Wash conditions were modified as follows: 4 washes for 20 minutes each in 1 × salts, 50% formamide at 47°C. 2 washes in NTE, 37°C, 30 minutes each; 1 wash in NTE plus 20 µg/ml Ribonuclease A (Sigma), 37°C for 20 minutes (salts and NTE as in Ingham et al., 1985), 2 washes for 10 minutes in PBS followed by incubation with anti-digoxigenin antibody at 1 in 750 dilution in PBS for 1 hour. Excess antibody was removed by washing 4 times for 20 minutes each in PBS followed by 3 times 5 minute washes in 100 mM Tris pH 9.5, 100 mM NaCl, 50 mM MgCl₂, 1 mM levamisole (Sigma), 0.1% Tween 20 (BDH). Detection of hybridisation carried out by adding 4.5 µl NBT, 3.5 µl X-phosphate (BCL nucleic acid detection kit) to 1 ml of above reaction buffer then placing a drop over sections and allowing colour reaction to develop for 30 to 40 minutes. Reactions were stopped by washing in PBS and allowing to air dry. Tissue was mounted in 85% glycerol, 12.5% water, 2.5% propylgallate and viewed with bright field illumination using a Nikon Microphot-FX.

Transformation constructs

All constructs were made using standard techniques and derived from the following genomic and cDNA clones, as shown in Fig. 3: pKScycA and pKScycB are 11kb *Bam*HI and 10kb *Sall* genomic fragments containing the complete cyclin A and B transcription units, cloned in the vector pKS Bluescript (Stratagene). The vector *NotI* and *Sall* sites on the 5' and 3' sides of the inserts were used for subcloning. 2.5kb cyclin A and 2.7kb cyclin B cDNA clones in the vector pNB40 (Brown and Kafatos, 1988) and 2.3kb cyclin B cDNA described below and subcloned as an *EcoRI* fragment in pKS Bluescript.

Construct ABB-1 consists of the following fragments: 4.6kb *NotI-NcoI* genomic fragment including the promoter plus 5' untranslated region of cyclin A; 1.7kb *NcoI-EcoRV* cyclin B cDNA coding region fragment and 1.2kb genomic *EcoRV-KpnI*

fragment containing the 3' untranslated of cyclin B plus approximately 400 nucleotides of non-transcribed sequence.

The AZB-1 construct utilises the same fragments containing the promoter plus 5' untranslated sequences of cyclin A and 3' untranslated of cyclin B. The coding region of cyclin B is replaced by that of the *E. coli* β -galactosidase gene from the vector pMC1871 (Pharmacia, Casadaban et al., 1983) by insertion of an *Nco*I linker into the unique *Sma*I site of the vector allowing excision of the 3.0kb β -galactosidase coding region fragment by digestion with *Sal*I, end-filling with T4 DNA polymerase and digestion with *Nco*I. BZB-1 construct made as above but using a 4.0kb *Nco*I fragment including the promoter plus 5' untranslated of cyclin B inserted into the *Nco*I site introduced into pMC1871. AZB-2 construct made as AZB-1, but using a 200bp *Dra*I-*Eco*RI cDNA fragment containing the 3' untranslated of the smaller cyclin B transcript.

All constructs were finally subcloned into the P-element transformation vector pW8 (Klemenz et al., 1987) and co-injected into *w¹¹¹⁸* embryos at 500 μ g/ml with the helper plasmid 2-3 at 200 μ g/ml (Laski et al., 1986) in injection buffer (0.1 mM NaPO₄, 5 mM KCl). Transformants were identified by their wild-type eye colour phenotype and single insert homozygous lines made by standard methods. For each of the ABB-1, AZB-1 and AZB-2 constructs single transformed line were examined, for BZB-1 two independent lines were examined.

Cloning and sequencing of the 2.3kb cyclin B cDNA

The cDNA was cloned from a library made from testes poly (A)⁺ RNA (gift of T. Hazelrigg) in the vector ZAP II. Positive clones were detected by hybridisation to an oligo-labelled probe made from the 1.6kb *Eco*RI-*Eco*RV cyclin B coding region fragment shown in Figure 2. A *Dra*I-*Eco*RI fragment containing the 3' end of the cDNA was subcloned into the vector pKS Bluescript and sequenced using a Sequenase kit (United States Biochemical).

Polymerase Chain Reaction amplification of RNA

Total RNA was prepared from approximately fifty pairs of hand dissected ovaries. These were homogenised in 100 μ l of lysis buffer (8 M urea, 0.35 M NaCl, 10 mM Tris pH 7.5, 10 mM EDTA) and extracted 3 times with 9:1 phenol:chloroform saturated with lysis buffer and once with chloroform. RNA was precipitated using 2 volumes ethanol, 0.1 volume 3 M sodium acetate pH 5.5 at -70°C for 1 hour, washed in 70% ethanol and resuspended in 50 μ l water. 5 μ l of RNA was used as template for first strand cDNA synthesis followed by PCR amplification (Kawasaki, 1990). The region between nucleotides 1840 and 2463 in the sequence of Whitfield et al. (1990) was amplified and the two PCR products corresponding to the 3' untranslated regions of each cyclin B transcript were subcloned into the vector pKS Bluescript and sequenced using a Sequenase kit (United States Biochemical).

Results

Germ-line transcription of the *Drosophila* cyclin genes

Previous studies suggested that the accumulation of cyclin B mRNA at the posterior pole of the egg must occur during oogenesis, since it is already concentrated in this region prior to fertilisation (Raff et al., 1990). In order that we might study the stage during oogenesis at which this concentration occurs we first undertook to examine cyclin A and B transcripts present in the ovaries of adult female flies (Fig. 1). A probe derived from a full-length cyclin A cDNA, detects a single ovary-specific transcript of 2.5kb (Fig. 1A,

lane 4) corresponding to the size of a cyclin A mRNA detected previously in early embryos (Whitfield et al., 1989). A fragment derived from the coding region of a full-length (2.7kb) cyclin B cDNA, detects two ovary-specific transcripts in adult female flies of 2.7kb and 2.3kb (Fig. 1B, lane 4). The 2.7kb transcript is female specific and corresponds to the major maternally provided RNA detected previously in 0 to 2 hour embryos, prior to the onset of zygotic transcription (Lehner and O'Farrell, 1990b; Whitfield et al., 1989). A 2.3kb transcript, previously thought to be male specific (Whitfield et al., 1989) is present in both adult males and females (Fig. 1B, lanes 3 and 4).

We find greatly reduced levels of each of these transcripts in the poly (A)⁺ RNA of adult males and females that are the progeny of a P-M dysgenic cross (Fig. 1, lanes 1 and 2 respectively on each filter). As these flies essentially lack testes and ovaries, respectively, we conclude that the majority of transcription of both cyclin A and cyclin B is germ-line specific in the adult. The continuing low levels of transcription detected may be due to incomplete penetrance of the dysgenic effect. In order to distinguish the forms of cyclin B transcript, we hybridised a northern blot filter with a cDNA fragment derived from the 3' end of the 2.7kb cDNA (the cDNA fragments used to make probes are shown in Fig. 2). This probe detects only the larger transcript present in females (Fig. 1C, lane 4). Hence we conclude that the shorter transcripts present in both males and females are missing sequences present in the 3' untranslated region of the longer transcripts.

We wished to isolate a shorter cyclin B cDNA from the longer cDNAs previously sequenced (Lehner and O'Farrell, 1990b; Whitfield et al., 1990). We therefore screened a cDNA library prepared from adult testes poly (A)⁺ RNA (see Materials and methods) using a probe prepared from the coding region of the 2.7kb cyclin B cDNA (see Fig. 2). The sequence (Fig. 2B) indicates that smaller transcripts can be generated by a splicing mechanism which must remove 393 nucleotides from the central region of the 776 nucleotide 3' untranslated of the larger 2.7kb transcript. The splice donor and acceptor sites are in accordance with consensus sequences determined by Mount (1982), and Burtis and Baker (1989), except for the presence of an adenine residue a position normally occupied by a pyrimidine (see Fig. 2). The two transcripts may also utilise differing lengths of poly (A)⁺ tail to fully account for the size differences observed.

We wished also to determine whether the smaller cyclin B transcripts present in adult female flies are generated in the same way. We therefore sequenced polymerase chain reaction (PCR) amplified segments from the 3' untranslated regions of the two cyclin B transcripts detected in adult females, using total RNA prepared from ovaries as template for first strand cDNA synthesis (see Materials and methods). The smaller transcript present in adult females was also found to be generated by the removal of 393 nucleotides from its 3' untranslated region.

Identification of a posterior pole localisation sequence in cyclin B mRNA

In order to define the sequences responsible for the posterior pole localisation of cyclin B transcripts, we con-

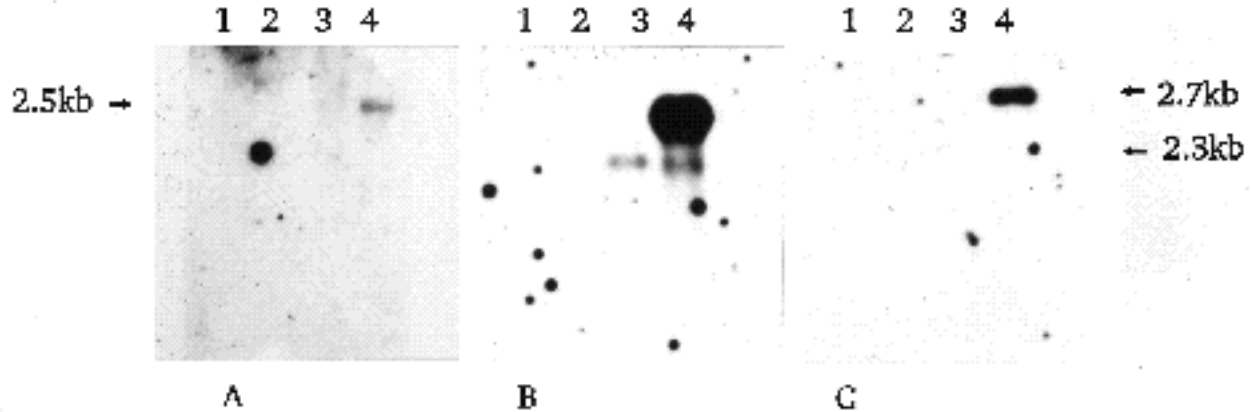


Fig. 1. Northern hybridisation showing transcripts detected in adult flies by cyclin A and B cDNA probes. Three identical northern blot filters were hybridised with probes made from (A) a 1.8kb *SalI*-*NotI* fragment derived from a 2.5kb cyclin A cDNA, (B) the coding region (1.35kb *EcoRV*-*EcoRI* fragment) and (C) the 3' untranslated (0.7kb *EcoRV*-*NotI* fragment) of the 2.7kb cyclin B cDNA shown in Figure 2A. On each filter, lane 1 contains poly(A)⁺ RNA from dysgenic males; lane 2, dysgenic females; lane 3, wild-type (Oregon R) males; and lane 4, Oregon R females. Transcript sizes in kilobases (kb) are indicated.

structed chimeric genes containing fragments of the cyclin A and B genes and the *E. coli lac Z* gene, referred to below as A, B and Z respectively. (see Fig. 3). We further distinguish the origins of the 3 cyclin B sequences as to whether they originate from genomic DNA, B-1, or the shorter cDNA, B-2. Hence we adopt a nomenclature in which a construct having the promoter plus 5' untranslated sequence of cyclin A, the coding region of the *lac Z* gene and a genomic fragment containing the 3' untranslated sequence of the cyclin B gene is referred to as AZB-1. We

used P-element mediated germ line transformation to generate transformed lines carrying these constructs. The distribution patterns of the chimeric RNAs expressed from these genes in transformed embryos were then examined by the whole-mount in situ hybridisation technique of Tautz and Pfeifle (1989; see Materials and methods) using anti-sense RNA probes corresponding to appropriate fragments from each construct underlined in Fig. 3.

The distribution of AZB-1 transcripts detected using a probe corresponding to the coding region of the *lac Z* gene

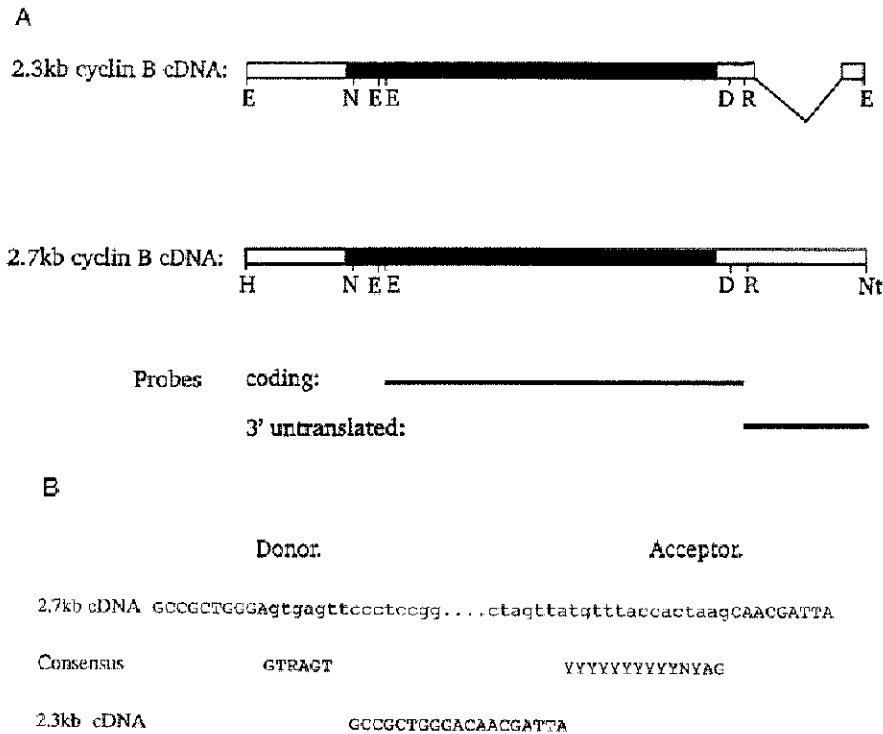


Fig. 2. (A) Structures of longer and shorter forms of cyclin B cDNAs. A 2.3kb transcript is generated by the removal of a 393 nucleotide (nt) region from the 3' untranslated region of the 2.7kb transcript. Underlined below the 2.7kb cDNA are the fragments used as probes to distinguish the two transcripts. Black and white boxes indicate coding and untranslated sequences respectively. D=*DraI*, E=*EcoRI*, H=*HindIII*, N=*NcoI*, Nt=*NotI*, R=*EcoRV*. (B) Relevant regions of the sequences of 2.3kb and 2.7kb cyclin B cDNAs. Splice donor and acceptor sequences in the 2.7kb cDNA are boxed. Sequences removed by splicing are shown in lower case letters and correspond to nucleotides 2004 to 2397 in the sequence of Whitfield et al. (1990). The non-consensus A residue in the splice acceptor sequence is underlined. Consensus donor and acceptor sequences (Mount, 1982) are shown below the corresponding regions of the 2.7kb cDNA. In the consensus acceptor sequence the length and composition of the pyrimidine rich tract is variable. The arrow indicates the splice junction in the 2.3kb cDNA. N=A,C,G or T. R=Purine, Y=Pyrimidine.

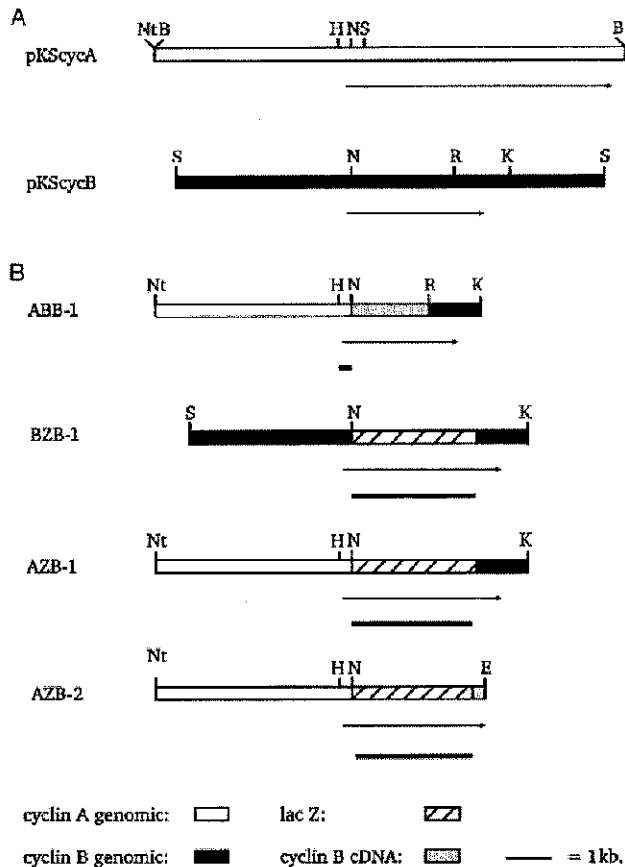


Fig. 3. (A) Genomic fragments containing the cyclin A and B genes. pKScycA and pKScycB are 11kb *Bam*HI and 10kb *Sal*I fragments including the complete cyclin A and B transcription units and the necessary promoter elements to direct transcription in oogenesis. (B) Chimeric cyclin gene constructs used to identify the localisation signal in the 2.7kb cyclin B transcript composed of cyclin A or B genomic DNA, the *E. coli lacZ* gene or cyclin B cDNA fragments as indicated in the key. A 3' segment derived from the cyclin B genomic sequence is referred to as B-1, whereas the 3' segment derived from the shorter cyclin B cDNA is referred to as B-2 (see text for details). Arrows indicate the transcribed regions of the cyclin A and B genomic fragments and of the chimeric genes. All constructs are aligned at their transcriptional start sites. DNA fragments used to make probes are indicated below each construct. These fragments were subcloned into pKS Bluescript and used to transcribe digoxigenin labelled antisense RNA probes. B = *Bam*HI, E = *Eco*RI, H = *Hind*III, K = *Kpn*I, N = *Nco*I, Nt = *Not*I, R = *Eco*RV, S = *Sal*I.

is shown in Fig. 4. Initially the message appears evenly distributed throughout the unfertilised egg (Fig. 4A), except for a concentrated cap at the posterior pole. The message in the body of the syncytial embryo becomes redistributed around the somatic nuclei as they migrate to the cortex in nuclear cycles 7-8 (Fig. 4B). The AZB-1 transcripts appear to be closely associated with the migrating nuclei in the manner of the endogenous cyclin B transcript rather than being simply excluded from the expanding yolk mass as observed for endogenous cyclin A transcripts (Raff et al., 1990). The message concentrated at the posterior pole becomes incorporated into the pole buds whose formation

is initiated in early cycle 9 as nuclei arrive at the posterior cortex. Following cellularisation, the bulk of AZB-1 transcripts are cleared from the embryo except for those in the pole cells (Fig. 4C) which remain detectable during germ band extension (Fig. 4D). Transcripts of the chimeric ABB-1 (not shown) and BZB-1 (Fig. 5D) genes have essentially identical distributions to those of the AZB-1 gene. They become incorporated into pole cells in nuclear cycles 8 to 9 where they are distributed on either side of the nuclei (Fig. 5, D, E, arrow indicates regions where RNA is most concentrated). They also show the peri-nuclear association typical of endogenous cyclin B RNA (Fig. 5, D, F). The only intact part of the cyclin B message retained in all of these chimeric genes corresponds to the 3' untranslated region. This suggests that it is these sequences that direct cyclin B RNA in its characteristic pattern.

We have confirmed this by examining the distribution of transcripts from a chimeric gene in which the shorter spliced form of the 3' untranslated region, derived from the 2.3kb cyclin B cDNA, replaces the genomic 3' end fragment used in the AZB-1 construct (Fig. 3, construct AZB-2). Transcripts of the AZB-2 gene are not concentrated at the posterior pole either in unfertilised eggs (not shown) or in the syncytial embryo (Fig. 5, A, B, arrow indicate pole buds). These transcripts also fail to show the peri-nuclear association, (Fig. 5, A, C) shown by the endogenous cyclin B message (Raff et al., 1990) in syncytial embryos. Instead the AZB-2 transcripts appear evenly distributed in the body of the syncytial embryo. Thus the deletion of part of the 3' untranslated sequence of the larger transcript must disrupt the sequences required for both posterior pole and peri-nuclear localisation.

Distributions of cyclin A and B transcripts in oogenesis

We have compared the patterns of the *Drosophila* cyclin A and B transcripts during oogenesis by in situ hybridisation using the coding regions of the genes as probes. Both genes are transcribed in the germarium (g; Fig. 6A, B). Here, in the anterior region, stem cells undergo mitosis to generate a daughter stem cell and a cystoblast which then undergoes four further mitotic divisions giving the sixteen precursor cells of the stage 1 follicle (reviewed by Mahowald and Kambyzellis, 1980). These cells differentiate, without further division, into fifteen nurse cells which undergo rounds of polyploidisation during stages 2 to 10 of oogenesis and the oocyte itself. Cyclin B transcripts unlike those of cyclin A, are clearly present at higher levels, in the presumptive oocyte, where they remain concentrated until stage 7 (Fig. 6B, arrowed in stage 7 follicle). Subsequently these transcripts appear to become dispersed in the cytoplasm of the oocyte as its volume increases (Fig. 6B, stage 8 follicle). The transcription of both genes then resumes in the nurse cells from stages 9 and 10 prior to the deposition of their contents into the oocyte at stage 12. Whole-mount in situ hybridisation shows that there is no suggestion of transcript accumulation at the posterior pole at this time (Fig. 6B, stage 10 follicle). We confirmed that cyclin B transcripts were present only in the nurse cells and not the oocytes of stage 10 follicles by in situ hybridisation to sectioned tissue (not shown). Transcripts enter the oocyte from the nurse cell complex at stages 12 to 13 (Fig. 7A) and become con-

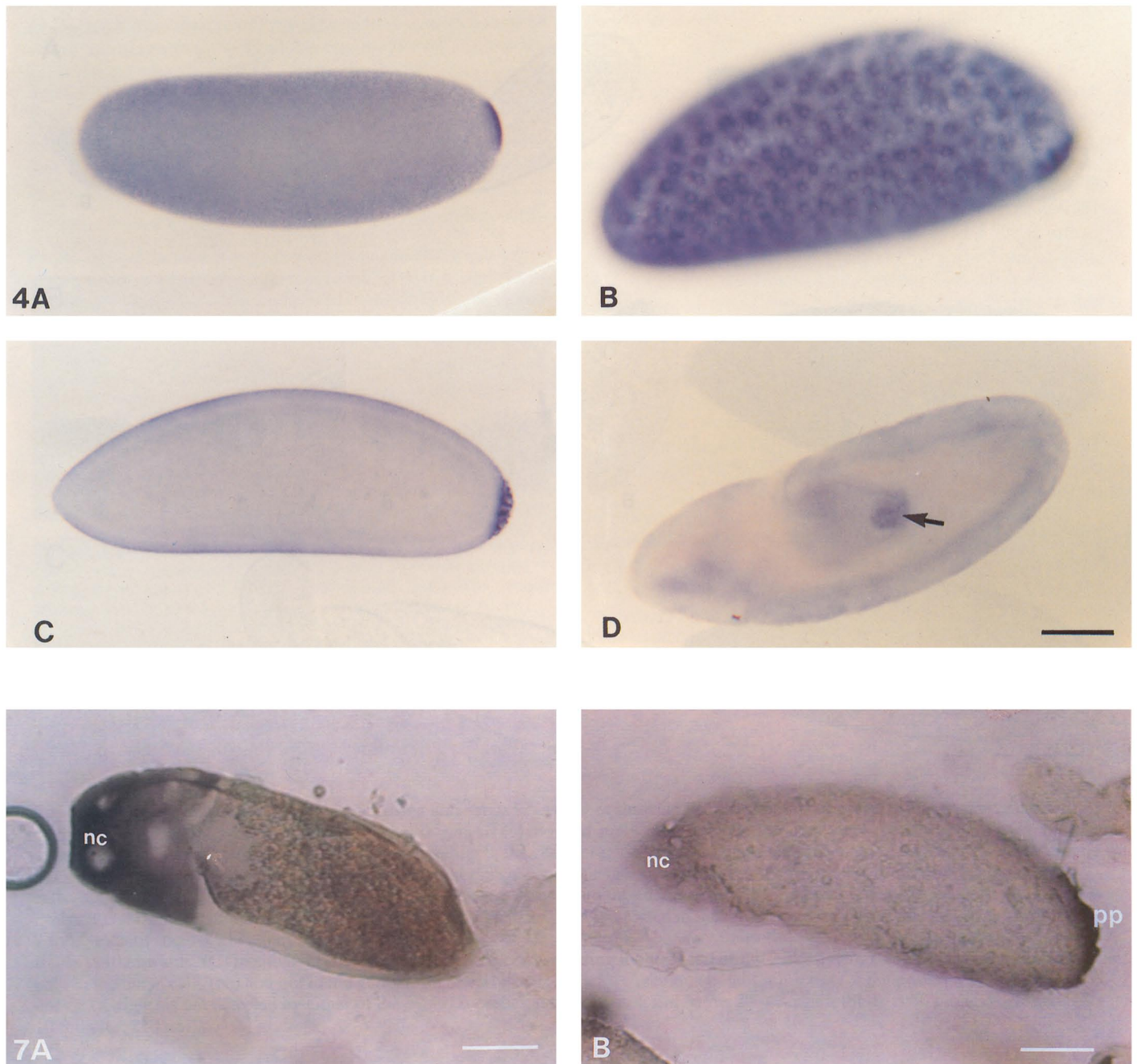


Fig. 4. Distribution of the chimeric AZB-1 RNA. In situ hybridisation to whole embryos with a digoxigenin-labelled antisense RNA probe corresponding to the *lac Z* coding region fragment. (A) unfertilised egg; (B) syncytial embryo at cycle 10 with transcripts concentrated around nuclei at surface of embryo. (C) newly cellularised cycle-14 embryo with RNA concentrated in pole cells to right; (D) germ band extended embryo with AZB-1 RNA present in pole cells, indicated by arrow. All embryos have anterior to left and posterior to right. Bar is 100 μ m.

Fig. 7. In situ hybridisation to sections through late stage follicles using an anti-sense RNA probe showing entry of cyclin B transcripts from the nurse cells (nc) into the oocyte at stages 12-13 (A). By stage 14 cyclin B transcripts have dispersed throughout the oocyte and are concentrated at the posterior pole (B, pp). Bar is 100 μ m.

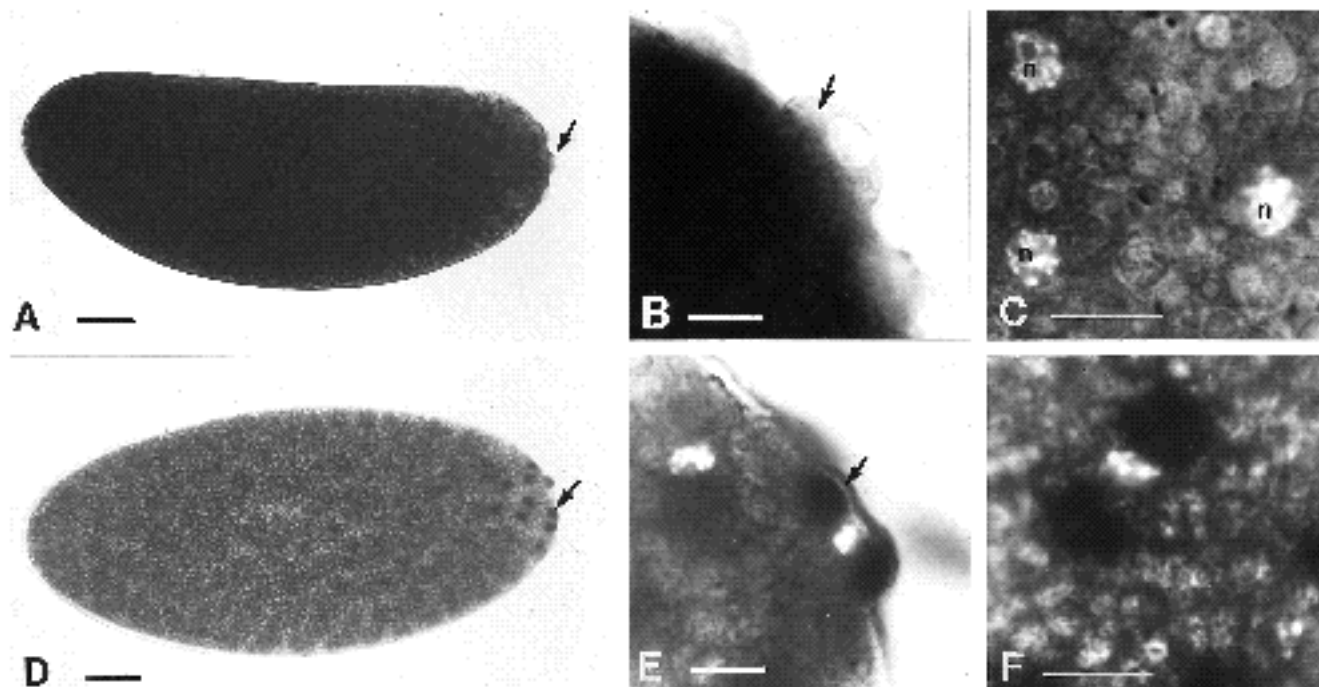


Fig. 5. In situ hybridisation to whole embryos showing the distributions of the chimeric AZB-2 (A-C) and BZB-1 (D-F) transcripts, detected using a *lac Z* coding region probe. The AZB-2 transcripts appear evenly distributed throughout the body of a cycle-10 embryo (A). They are not detected in the pole buds (C). The arrows indicate those regions of pole buds in which cyclin B transcripts are normally concentrated. The AZB-2 RNA also fails to show perinuclear association (C; n indicates nuclei). The BZB-1 transcripts are both concentrated at the posterior pole (D,E; arrows indicate regions where RNA is most concentrated) and show peri-nuclear association (F). DNA is visualised in B, C, E and F with staining with Hoechst 33258, dark staining indicates RNA. Bars in A and D are 100 μ m, in B, C, E and F, 10 μ m.

centrated at the posterior pole during stages 13 to 14 (Fig. 7B).

We wished to determine whether the two forms of the cyclin B transcript showed any differential distribution during oogenesis. We therefore examined the in situ hybridisation pattern given by a probe made from the 3 untranslated sequence of the 2.7kb cyclin B cDNA, with that of a probe made from the cyclin B coding region (see Fig. 2A for details of the probe used) in order to detect only the larger cyclin B RNAs. We were able to detect transcripts only in the nurse cell complex from stages 9 to 10 onwards and not in the posterior germarium (g; Fig. 6C) or early stage oocytes. This contrasts with the ability of the coding region probe to detect both patterns of cyclin B transcript distribution (Fig. 6B). Hence it appears that the smaller form of the cyclin B message is preferentially synthesised in the early stages of oogenesis. Restricted synthesis of the larger message in the nurse cell complex (Fig. 6B, C) is consistent with the previous finding that this is the only maternal cyclin B transcript that can be detected in 0 to 2 hour embryos by Northern blotting (Whitfield et al., 1989). The cyclin B RNA that is present at the posterior pole appears therefore to be both synthesised and localised late in oogenesis and thus follows a distinct pattern of expression from that seen in the earlier stages. We are unable to detect intermediate stages of cyclin B mRNA accumulation during oogenesis suggesting that structures

able to specifically recognise and capture this message might already be positioned at the posterior pole.

Discussion

We have compared the distributions of the *Drosophila* cyclin A and B transcripts in oogenesis and find both to be present in the cells of the germarium, consistent with a requirement for cyclins in that region of the ovariole, in which mitosis takes place. In subsequent stages of oogenesis the pattern of accumulation and redistribution of cyclin B transcripts is more complex than that of cyclin A. Only cyclin B transcripts are seen in the presumptive oocyte in the early stages of oogenesis. These are the shorter forms of the cyclin B RNA that lack a segment of the 3 untranslated sequence present in the transcripts produced at later stages. These transcripts persist in the oocyte until stages 7 to 8. From stages 9 to 10 onwards both cyclin A transcripts and a larger form of the cyclin B transcript accumulate in the nurse cells prior to the deposition of their contents into the oocyte in stages 11 to 12 of oogenesis. Cyclin B transcripts are then accumulated at the posterior pole in stages 13 and 14. This is consistent with previous findings that cyclin B transcript accumulation is disrupted in eggs of females homozygous for the mutations *cappuccino*, *spire*, *staufen* and *oskar* (Manseau and Schupbach, 1989; Schup-

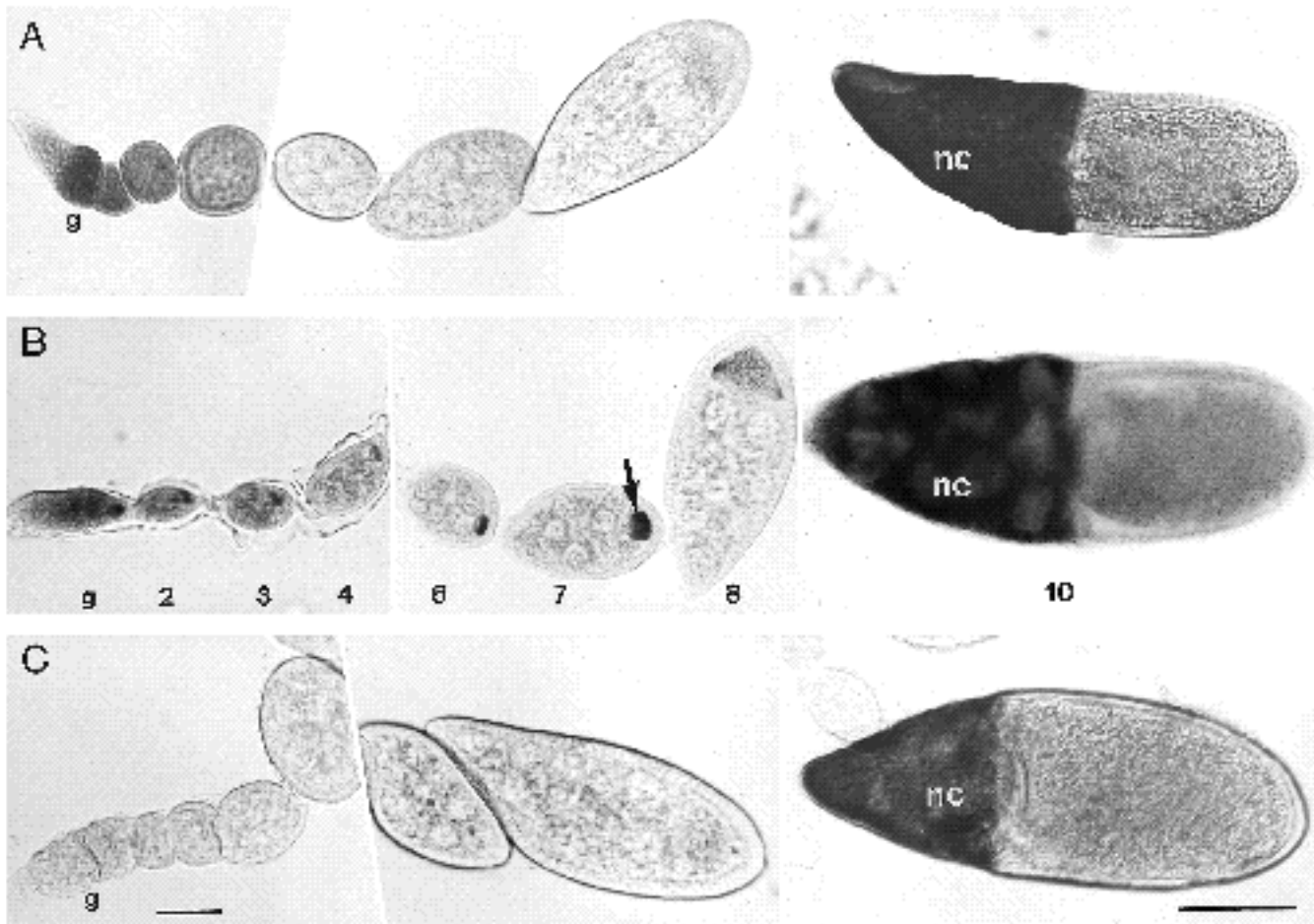


Fig. 6. In situ hybridisation to whole ovaries using anti-sense RNA probes showing the distributions of cyclin A and B transcripts during oogenesis (see Materials and methods). Stages of oogenesis (Mahowald and Kambyzellis, 1980) are indicated below the follicles shown in panel B. (A) Hybridisation with a probe corresponding to the coding region and 3' untranslated sequences of the cyclin A cDNA shows transcripts present in the posterior germarium (g) but not in subsequent stages of oogenesis, until the onset of transcription in the nurse cells (nc) at stages 9-10. (B) Hybridisation with a probe corresponding to the coding region and 3' untranslated sequences of the 2.7kb cyclin B cDNA detects transcripts in the germarium (g) which continue to be seen in the presumptive oocyte (indicated by arrow in stage-7 follicle) until stage 8 when they appear to disperse in the oocyte cytoplasm. Abundant transcripts are detected in the nurse cells (nc) in stages 9-10 onwards. (C) In situ hybridisation using a probe corresponding to the 3' end of the 2.7kb cyclin B cDNA detects transcripts only in the nurse cells (nc) and not in the germarium or early stage follicles. Accumulation of transcripts at the posterior pole of the oocyte (to right) is not detected by either of the cyclin B probes at these stages. Bar below early stage follicles is 50 µm and below stage-10 follicles, 200 µm.

bach and Wieschaus, 1986; Lehmann and Nusslein-Volhard, 1986). These mutations also disrupt the accumulation of *vasa* protein at the posterior pole which normally occurs at stage 10a of oogenesis (Hay et al., 1988; Lasko and Ashburner, 1990). This suggests that the accumulation of cyclin B transcripts may be dependent upon their interaction with components of the polar granules already positioned at the posterior pole.

We have shown that the complete 3' untranslated sequences of the larger form of the cyclin B transcript direct them to the posterior pole during oogenesis. Two other maternally provided RNAs in which localisation signals have been identified are those encoding the anterior morphogen *bicoid* (Macdonald and Struhl, 1988), and the dorso-ventral determinant K10 (Cheung et al., 1992). *bicoid* RNA is concentrated at the anterior pole of unfertilised eggs

and its accumulation has been shown to occur in several phases during stages 6 to 12 of oogenesis (St.Johnston et al., 1989). K10 transcripts however appear to be selectively transported into the oocyte from as early as stage 2 of oogenesis and they accumulate at its anterior margin (Cheung et al., 1992). Both the initial localisation patterns and the structures involved appear therefore to be distinct for each of these RNAs. All however are dependent on sequences present in the 3' untranslated of the transcript. Examination of the 3' untranslated regions of both the *bicoid* and K10 transcripts reveal a number of long inverted repeats predicting that these regions are capable of forming extensive secondary structures. The significance of this in directing the localisation of these transcripts is uncertain but similar examination of the sequence of cyclin B transcripts predicts that it is incapable of forming such struc-

tures. Primary sequence may therefore be of greater importance in directing the localisation of this RNA.

In addition to being concentrated at the posterior pole, cyclin B transcripts in the body of the embryo become associated with the nuclei as embryonic development proceeds. As this association is disrupted by microtubule destabilising drugs, it has been suggested that either nascent cyclin B polypeptides or polysomes might be associating with microtubules and dragging the RNA with them, or that the cyclin B RNA itself is targeted to microtubules (Raff et al., 1990). Our finding in this paper supports the latter explanation, since the 393 nucleotide 3' untranslated sequence of the larger maternal cyclin B transcript required for posterior accumulation of the mRNA is also required for its perinuclear localisation in the body of the syncytial embryo. It is possible that either discrete sequence elements within the 3' untranslated region are responsible for the two patterns of localisation or that the same sequence is targeting the RNA in both processes. This can only be resolved by further dissecting the region using the type of experimental approach we have described.

Although it was previously postulated that two distinct mechanisms are responsible for the two patterns of localisation observed, the two processes may share a common intermediary. Such a molecule could be associated with the astral microtubules nucleated by centrosomes. This is possible since the polar granules are also observed to cluster around centrioles in a number of *Drosophila* species (Rabinowitz, 1941; Counce, 1963; Mahowald, 1968). Furthermore, pole cell formation itself appears to be triggered by an interaction between a pole plasm component and centrosomes, or a molecule associated with the centrosomes (Raff and Glover, 1989). There is currently no indication of which polar granule component could interact with centrosomal microtubules and so it must remain a matter of speculation as to whether this component could also be present associated with astral microtubules in the body of the embryo. It is feasible that such a molecule could form part of a cytoskeletal network required for the transport of posterior pole components from the nurse cells. The mechanism of this transport is not well understood but it has been suggested that the products of the *capu* and *spir* genes, needed for both anterior-posterior and dorsal-ventral patterning, may contribute to such a network required to localise morphogens (Manseau and Schupbach, 1989).

The functional purpose of targeting cyclin B transcripts to either the peri-nuclear regions of the syncytial embryo or the posterior pole of the developing oocyte remains uncertain. A possible explanation for the former pattern of localisation could be to help direct cyclin B protein to its site of action, prior to it becoming complexed with p34^{cdc2}. We presume that the cyclin B transcripts at the posterior pole of the embryo will be translated later during the development of the germ line. They persist in the pole cells throughout germ band extension (Whitfield et al., 1989), but there appears to be no increase in the level of cyclin B protein detectable by immunofluorescence in pole cells, above that of the surrounding cells, at least until cycle 15 (G. Maldonado-Codina, personal communication). Furthermore when cyclin B transcripts are detected in the oocyte during stages 2 to 7 of oogenesis, cyclin B protein

is undetectable in this cell by immunofluorescence (B.D. unpublished observation) although it is present in the unfertilised egg (Whitfield et al., 1990). Hence translational repression of cyclin B may be functioning both in oogenesis and later in embryogenesis. Detailed examination of the fate of cyclin B mRNA in the pole cells and the developmental regulation of its translation should assist in elucidating the possible role of cyclin B in the proliferation of these cells.

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